

BIOLUMINESCENT BEHAVIOR IN *RENILLA*. I. COLONIAL RESPONSES ¹

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The colonial soft coral (alcyonarian) *Renilla*, or "sea pansy," consists of a flat, bilobed, nearly circular platform or rachis, which normally rests on the ocean bottom, and a central holdfast or peduncle which extends down into the sand from the under surface. From the upper surface of the rachis project several thousand zooids, which are of two kinds: larger retractile feeding polyps or autozooids, which occur singly, and much smaller, modified individuals called siphonozooids, which occur in clusters and function as intake pores for the sea water which circulates through the spongy tissue of the rachis and peduncle. The colony undergoes peristaltic muscular movements and periodic deflations and expansions (Parker, 1920a; Hyman, 1940).

The luminescence of *Renilla*, which, in the words of Agassiz (1850, page 209), "shines at night with a golden green light of a most wonderful softness," has long stimulated the curiosity of zoologists. The colony does not ordinarily luminesce spontaneously under laboratory conditions: rather, the light is manifested as a wave or series of waves radiating over the rachis from any point of stimulation. The polydirectional and non-decremental spread of luminescence have been assumed to mean that an unpolarized nerve (neuroid) net is present in the rachis.

Using mainly mechanical stimulation, Parker (1920b) defined many of the basic properties of the luminous response in *Renilla*. More than thirty years later his observations were extended by me (Buck, 1953, 1955) and particularly by Nicol (1955a, 1955b), using electrical stimulation. The present paper gives some new anatomical details and amplifies such of my visual behavioral observations as were not superseded by Nicol's photometric recordings.

MATERIAL AND METHODS

Renilla köllikeri (miscalled *R. amethystina* by Parker) from two Californian sources was used. For the bulk of the visual work colonies were dredged weekly from a depth of 75 feet in the mouth of Newport Harbor in June and July and stored in running sea water at 19-24° C in aquaria exposed to diffuse daylight. Supplementary observations were made on colonies brought up by divers from Los Angeles Harbor in March and stored in refrigerated aquaria. As will appear, and as noted also by Nicol (1955a), the behavior of "summer" and "winter" pansies differed strikingly in certain respects. Since all colonies contributed to understanding zooid and colony behavior, both populations were accepted as normal

¹ Dedicated to Professor Curt Stern of the University of California for his 70th birthday.

without attempting to ascertain the cause of the differences or whether they are in fact consistently seasonal or geographically distinct.

For visual observation colonies were studied at magnifications up to $100\times$ in four-inch finger bowls of standing sea water. Since it is known that ambient light can inhibit luminescence, colonies were dark-adapted for at least a half hour before testing.

Colonies were stimulated *via* condenser shocks of 100 msec duration and nominal strengths up to 80 volts. Click-stop control knobs enabled stimulus parameters to be set in total darkness. The electrode consisted of two strands of no. 24 enameled

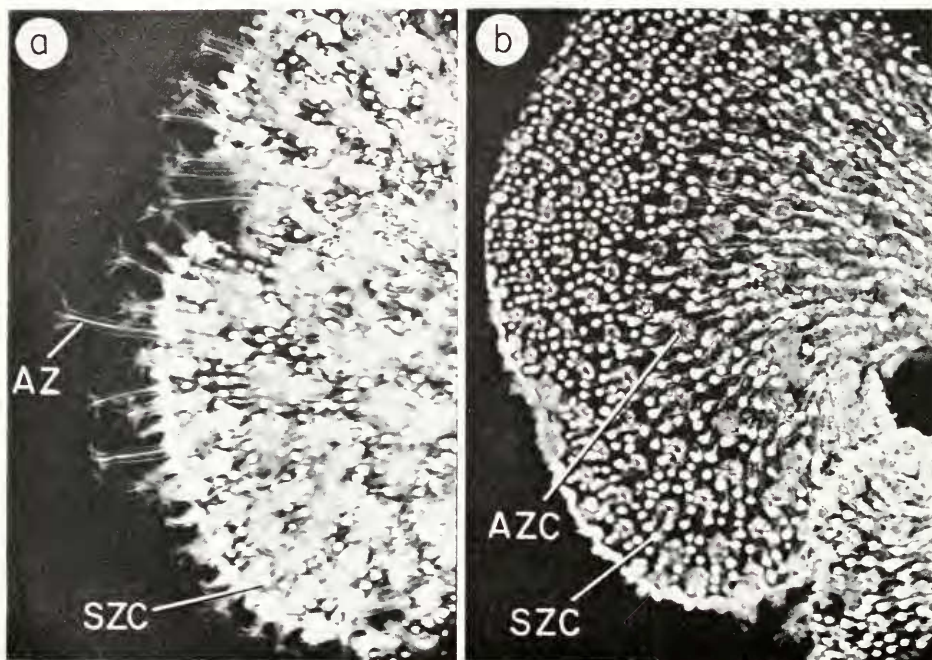


FIGURE 1. Portions of living *Renilla rachs* in sea water, $\times 3$; (a), expanded colony; (b), contracted colony; SZC, siphonozoid cluster; AZ, expanded autozoid polyp; AZC, calyx or base of autozoid.

copper wire sealed in a glass tube except for the tips, 3 mm apart. The bare metal was cleaned periodically with a carborundum stick to reduce fouling by mucus. The electrode was carried on a sliding rack so that it could be lowered to the rachs in total darkness by feel. The electrode was usually positioned about a third of the distance in from the margin of the rachs to the center and was pressed firmly into the surface to prevent shifts in position due to peristalsis or deflation. Voice descriptions of behavior were tape-recorded concurrently with the stimulus signals. Water temperatures varied between 20° and 25° .

Since the absolute intensity of *Renilla* luminescence is low, care was taken to dark-adapt the eyes for at least 20 minutes before beginning observations.

Attempts were made to detect local responses by recording through a small hole in an opaque screen covering the rachis and by using a 1.5 mm diameter light guide. Technical details of the oscilloscopic records and image intensifier photographs presented will appear in a following paper.

RESULTS

Morphology

The colony is quite muscular and a large rachis can vary in diameter between 10 cm when fully distended with water to as little as 5 cm when maximally contracted. Independently of the contractility of the rachidial tissue itself, the autozoid polyps (AZ, Figs. 1, 2) are able, by shortening the tentacles and trunk and turning the trunk inside out, to retract completely into the interior of the rachis so that only the star-shaped, saucer-like calyx remains above the rachidial surface (Figs. 1b, 2b). The siphonozoid clusters, however, do not change shape appreciably even on exposure to fixing solutions (Figs. 1, 2). The clusters often lie along radii reflecting the mode of colony growth (Wilson, 1884). The autozooids are more closely spaced near the outer margin than in the interior of the rachis (Fig. 1b). In a large rachis there may be 400–500 autozooids and 1500–2000 siphonozoid clusters comprising perhaps 15,000 individual polyps. Areal distributions of the 442 autozooids and 1456 siphonozoid clusters on a preserved rachis 55 × 65 mm are given in Figure 4. In this specimen the average number of siphonozoids per cluster was about 6.

The autozooids have eight pinnate tentacles (Fig. 2a). These are of equal size except in autozooids at the extreme margin of the rachis, where two are typically larger than the others. The calyx has five lobes, representing preferential development of interradial regions of the basic octomeroous plan (Fig. 2b). The siphonozoid clusters consist of a single, somewhat larger "principal siphonozoid" with two vestigial tentacles (VT, Figs. 2a, 3) surrounded by 4–15 smaller ovoid individuals (Fig. 3). The autozoid calyx lobe tissue contains granular material that is white by reflected light. Similar refractile material occurs in two parallel narrow strips in each autozoid tentacle and in the siphonozoids.

Particularly when the mucus feeding canopy (MacGinitie and MacGinitie, 1968) is stripped off the rachis it can be seen that the colony surface is thickly strewn with minute aciculate spicules, usually deep blue but occasionally amber or even colorless (SP, Fig. 2b, 3). Spicules are also banked up around the bases of the autozooids and the siphonozoid groups, like tartar around a molar tooth. The minority of colonies in which the spicules are amber rather than blue have, when expanded, a predominantly pink overall rachidial color as compared with the uniform purplish color of specimens with only blue spicules, and more prominent radii. No difference in luminosity between the two types of pansy was observed.

Sites of luminescence

Luminescence in *Renilla* is not as simply localized as might be expected. In Newport specimens the first touch usually caused an immediate retraction of the autozooids which lasted at least half an hour even without further stimulation.

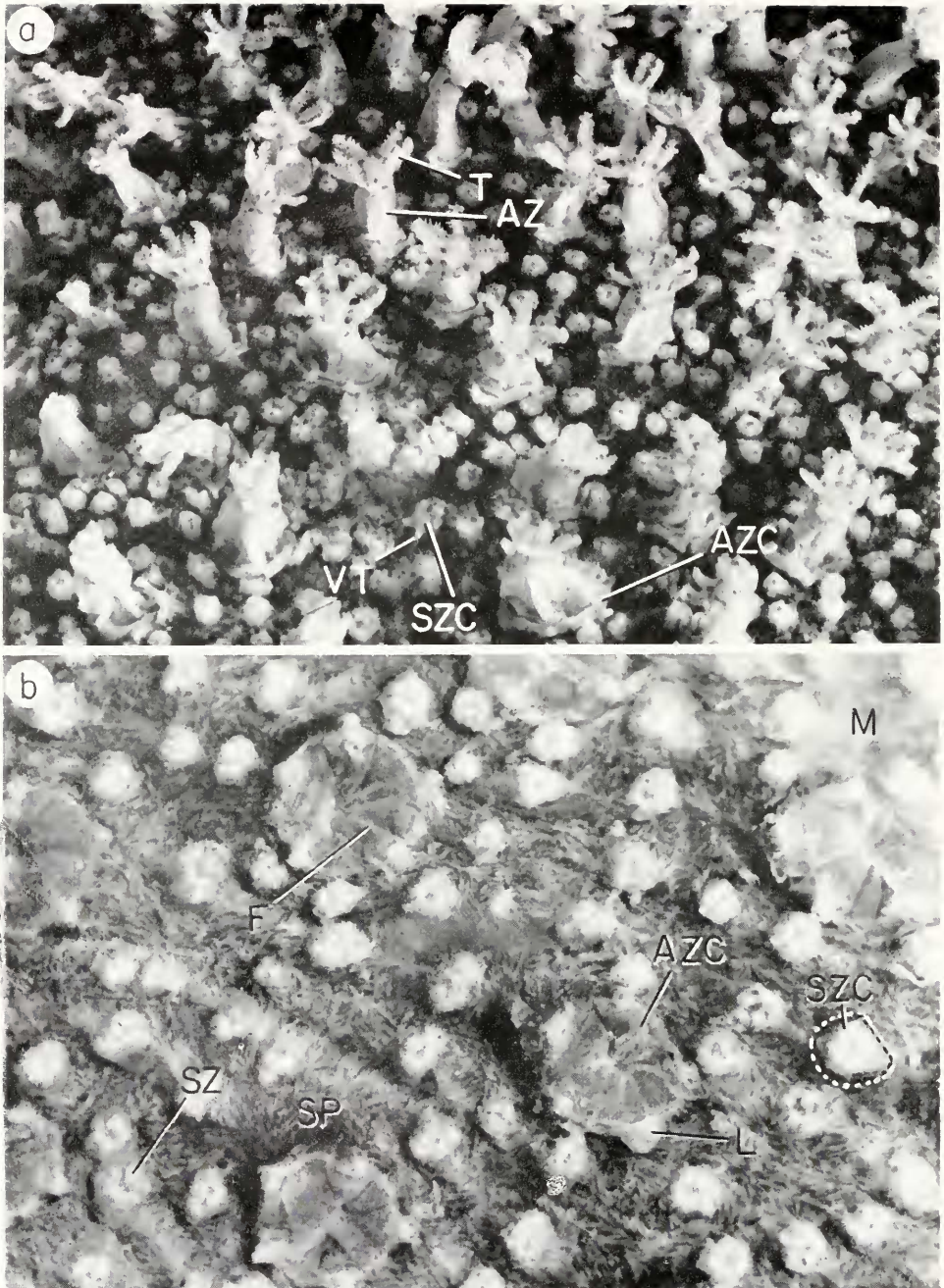


FIGURE 2. Surface views of rachides fixed by sudden flooding with hot formalin, $\times 10$; (a), partly expanded colony; (b) contracted colony; AZ, autozooid with eight pinnate tentacles (T); SZ, one of several siphonozooids of a cluster (SZC); SP, spicules. In a, one

When such a summer rachis was stimulated, small, circular, dark islands were seen among the multitude of spots that lit up briefly as the response wave swept across the colony (Fig. 5). By using very dim incident light during the response it was seen that each luminous spot was a siphonozooid cluster whereas each dark area contained a retracted autozoooid.

Since the autozoooids retracted upon touch in summer specimens it was not known whether they participated in the luminous waves that occur with moderate and infrequent stimulation but at least the calices were non-luminous. In summer pansies in long-stagnant water, in which the autozoooids remained extended after stimulation of the rachis, the autozoooid trunks and tentacles were not luminous

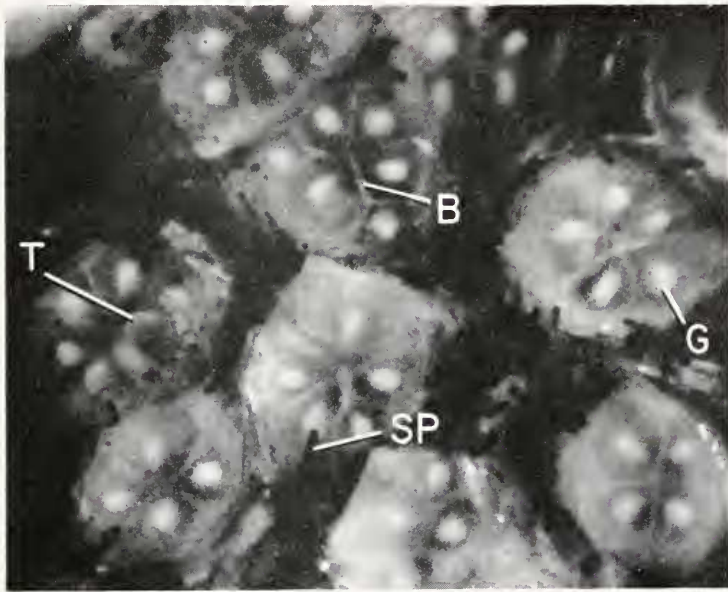


FIGURE 3. Nine siphonozooid clusters of fixed, contracted rachis, $\times 40$; B, polygonal boundaries between individual siphonozooids; G, shrunken gastroderm; T, vestigial tentacle; SP, spicule.

though the siphonozooids still responded. However, in normal pansies undergoing strong repetitive stimulation the autozoooid calices did begin to produce light. This light was considerably dimmer and much longer lasting than that from siphonozooid clusters and seemed concentrated near one or more of the five lobes of the calyx.

Winter pansies from Los Angeles differed from Newport summer animals in (a) not immediately withdrawing their autozoooids upon stimulation, (b) often being poised in a hyperexcitable state such that a single touch led to a brief and spectacular general luminescence after which the colony was almost inexcitable for

cluster shows the two vestigial tentacles (VT) of its principal siphonozooid. In b the mucus feeding canopy (M) has been stripped from most of the surface. Calices of involuted autozoooids (AZC) have five fleshy lobes (L) and enclose folds (F) corresponding to the eight internal mesenteries of the column.

a long period, and (c) having autozooids which were not only luminous but brilliantly so. Due to simultaneous siphonozooid activity it was difficult to be sure that the stalks or columns of these extended autozooids were entirely non-luminous, though they appeared to be so, but there was no doubt that each crown of tentacles gave flashes fully as sharp and bright as those of a siphonozooid cluster. In fact, as seen in a localized region under the microscope, the autozooids gave the luminous wave a striking bimodal character. The first peak represented the typical siphonozooid activity on the rachidial surface, and was followed by a relatively dark period of a quarter to half second, presumably representing the

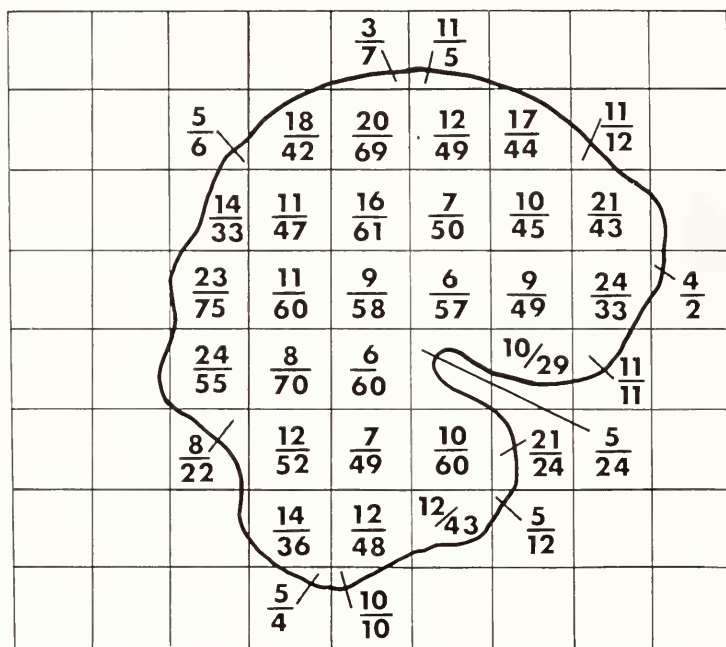


FIGURE 4. Relative distributions of 442 autozooids and 1456 siphonozooid clusters over fixed 55 x 65 mm largely contracted rachis. Note AZ/SZC ratios varying from up to 1:1 at the margin to 1:10 in the center.

time required (at the 10–15° temperature of the sea water in which the colonies were originally examined) for the excitation to climb the centimeter-long, apparently non-luminous autozooid columns, after which a second peak occurred as light fairly blazed up in the tentacles and ran to the tips.

The response characteristics of the winter animals persisted during 24–36 hours' exposure to water of 20–25°, hence seemed not to be a temporary response to low temperature.

At 20 to 30 diameters magnification the light of *Renilla* seemed to come principally from the neighborhood of the whitish refractile substance within the siphonozooids, the lobes of the autozooid calyx and the strips along each autozooid tentacle. By analogy with other bioluminescent organisms it would be expected

that the actual photogenic tissue is translucent and that its apparent association with the refractile material is simply due to enhancement of the luminescence by reflection. Luminous material was never found in the ambient water or on utensils or hands, even after considerable handling of the rachis. One could easily be deceived on this point, however, if employing injurious stimulation such as stabbing

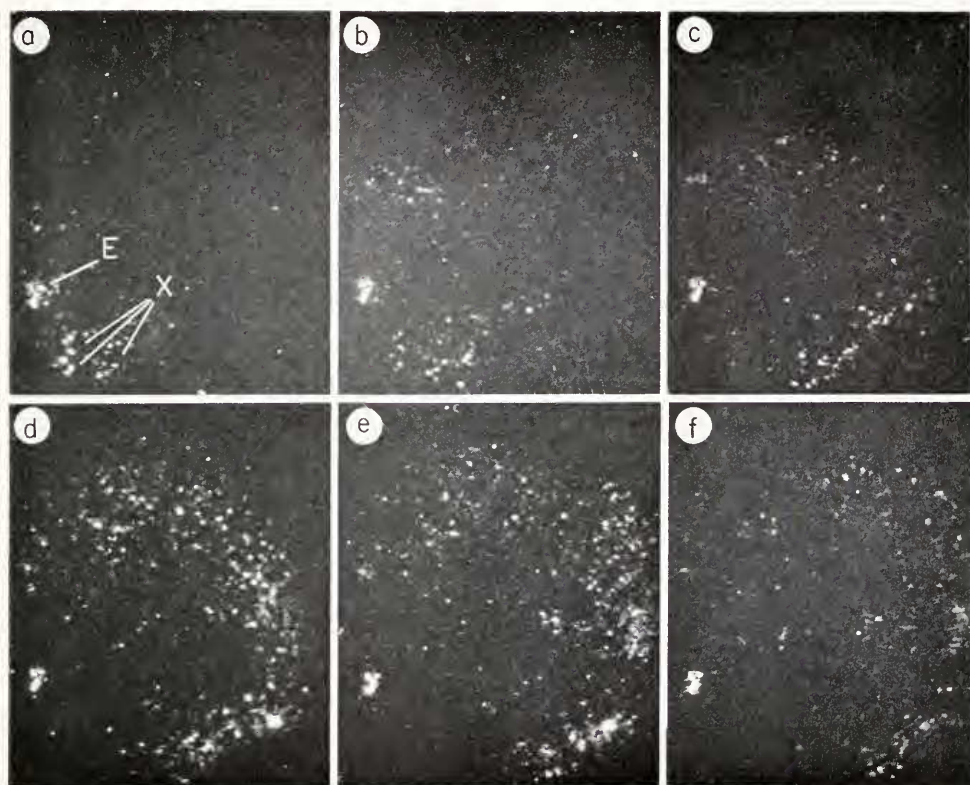


FIGURE 5. Six stages in passage of a luminous wave from the point of electrical stimulation (E) across a *Renilla* rachis from left to right. Panels are, respectively, the 1st, 9th, 17th, 25th, 33d and 41st frames of a 16 mm cinema film exposed at 16 frames/sec in photographing the phosphor of an image intensifier tube, $\times 3$. The "X"s in panel a indicate presumed sites of non-luminous autozooids amid circles of luminous siphonozooid clusters. Several such dark circular islands are visible also in the 11 to 1 o'clock and 4 to 6 o'clock sectors of panel c and in the 11 to 2 o'clock sector of panel d.

with a needle, since small bits of luminous tissue readily become lodged in the communal mucus feeding canopy. Spawning male colonies could also be deceptive since the sperm-filled surrounding water assumes a milky luminosity when illuminated by flash or glow. The ability to flash for long periods without significant fatigue (see below) is an additional indication of intracellular luminescence.

Effects of ambient light and of stagnation on excitability

Working on summer pansies I confirmed reports (Parker, 1920b; Nicol, 1955a; Kreiss and Cormier, 1967) that ambient daylight inhibits luminescence and found also that light adaptation raises the threshold for stimulation. However, in contrast to the inhibitory effect of diffuse daylight, pansies exposed for 5–30 minutes to direct sunlight, though drastically contracted, showed low threshold and bright luminescence upon stimulation and even a tendency to become autoexcitable. The temperature rise in the water did not exceed 2° during such exposure.

Stagnation of water in the finger bowls in the dark had no effect up to 8 hours, but thereafter increasing numbers of colonies showed inflated rachides, permanently extended autozooids, induced spawning, higher thresholds and dim luminescence, the first three effects showing also in colonies kept in the light. However, *Renilla* usually survives 24–36 hours in a finger bowl at 20–22°.

Types of luminous response

As will appear, *Renilla* has a large repertory of luminous emissions which vary with both physiological state of the colony and intensity of stimulation. The simplest of these is the single wave evoked by weak electrical stimulus, which I will call the "normal" wave (Fig. 5). With the equipment used in this study the threshold stimulus, using 100 msec shocks and fresh, dark-adapted summer pansies, was about 4 V.

The normal wave. I confirmed previous descriptions of response waves as spreading radially, non-decrementally and with the leading edge of the wave brightest. I also found that the breadth of a wave—that is, the number of ranks of zooids participating at any instant—differed widely in different specimens and under different conditions. Curiously, the luminescence did not usually appear to originate at the point of electrode contact; rather, the electrode seemed surrounded by a circular dark zone 5–10 mm in diameter out of which the waves became visible.

The velocity of excitation spread across the rachis was in the 4–7 cm/sec range at 20–25°. Though waves usually moved with uniform velocity the impression was occasionally gained of local variations in conduction velocity, particularly in rachidial strips (Parker, 1920b) in which the total path for passage of a wave can be increased from about 7 to 30 cm (Figs. 6a, 6b). A rare phenomenon seen also in such strips was an apparent "fraying out" of the originally single wave into two or more by the time it reached the end of its travel.

As observed also by others, it is the almost invariable rule that a luminescent wave, once initiated, sweeps across the entire rachis with no decrement in velocity or intensity. This is true of waves induced by barely adequate stimulation as well as those induced by intense shocks, and of dim waves as well as the most brilliant. In fact, rather than dying out, very dim waves sometimes first become noticeable at points well removed from the electrode and seemed to brighten a little during their passage. Out of thousands of waves observed I have seen only a half dozen failures to affect the whole rachis and most of those involved dimly luminous pansies, long resident in darkness, in which secondarily non-luminous but still conducting regions might have been involved. In a single pansy that had been ex-

posed to direct sunlight, however, decremental conduction seemed to be shown clearly. In this specimen, which, contrary to the usual effect of sunlight, had a high threshold and was consequently being stimulated with high voltage and at a

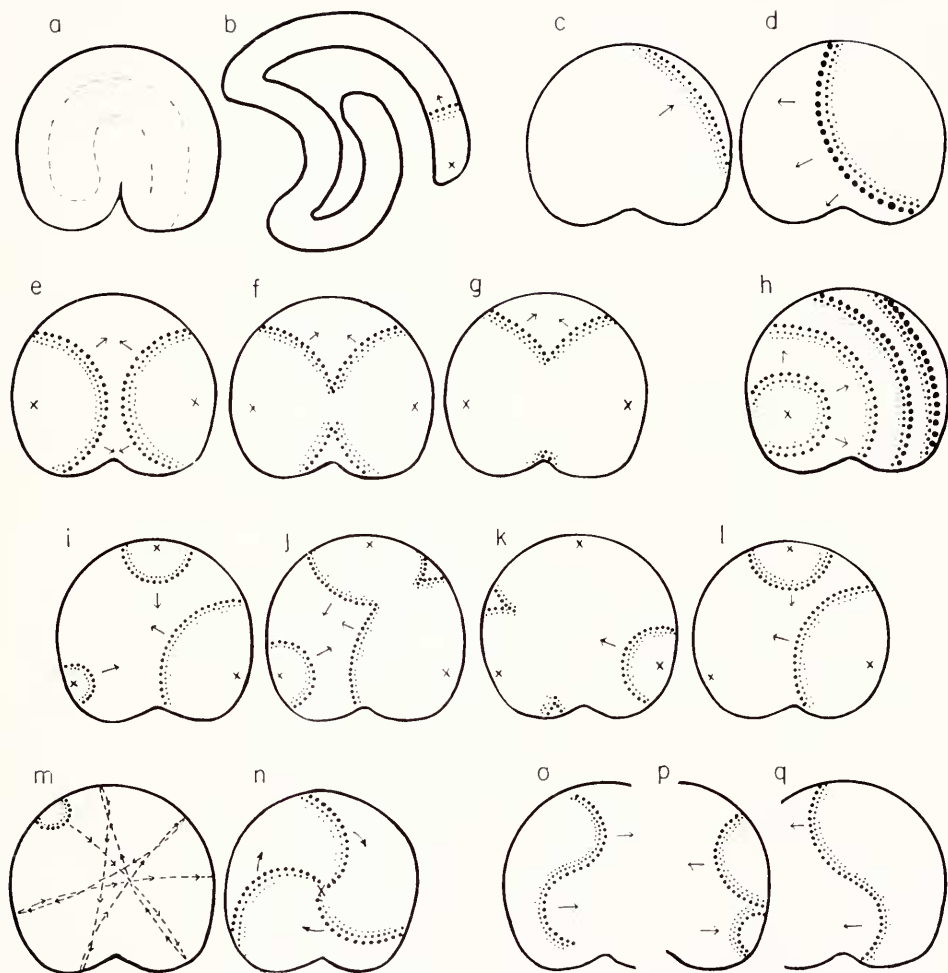


FIGURE 6. Diagrams showing various luminosity phenomena of *Renilla* rachis: a, b, method of cutting rachis to yield a strip with approximately quadrupled conduction path; c, right-moving luminous wave about to die out at rachidial margin in 2 o'clock sector; d, left-moving wave which originated spontaneously at about point where wave diagrammed in Figure 6c died out; e, f, g, three stages in collision and mutual cancellation of two luminous waves originating simultaneously from two electrodes (X) (see text); h, volley or family of luminous waves induced by mechanical stimulation at X (see text); i, j, k, l, four successive stages in a type of frenzy with several excitation centers, giving the impression of "boiling" luminescence (see text); m, simple type of frenzy in which an apparently single luminous wave bounces back and forth across the rachis (see text); n, type of frenzy giving the impression of a rotating luminescent "propeller"; o, p, q, type of frenzy in which a wave may have a front combining both convex-forward and concave-forward segments (o) which then reverse upon "reflection" (q).

frequency of 1/sec, the response began locally with the 6th shock, increasing in brightness and spreading farther out until at the 10th shock the whole rachis was involved.

Conduction rate in relation to possible tissue path was compared in broad and narrow strips. For example, a strip 12 mm wide and 90 mm long was split nearly to one end into strips 2 and 10 mm wide: A stimulus at the junction point elicited waves that were conducted at the same rate (4 cm/sec) in both strips. This suggests that if there are individual connections within the rachidial tissue these are numerous enough that a very direct conduction path can be utilized between any two points.

Facilitation and adaptation. Typically *Renilla* did not respond to the first few of a series of near-threshold stimuli repeated once every two seconds, and usually, after beginning to respond, produced several successively brighter waves until a plateau of wave intensity was reached. If stimulation was continued, responses usually began to be skipped, at first occasionally and then more and more frequently until the colony ceased to respond. A modest increase in stimulus strength then ordinarily reinstated response for another period, after which adaptation again supervened. The existence of effector facilitation is also suggested by the succession of progressively less frequent and less bright spontaneous waves which often followed the cessation of intense stimulation ("defacilitation").

Neuroeffector facilitation in autozooid calices is to be inferred from the brightening of the coarse-grained background glow that often paralleled the increase in intensity of the fine-grained siphonozooid flashes during serial stimulation, but even among winter pansies the autozooids did not remain extended during stimulation consistently enough to permit conclusions about possible facilitation of luminescence in tentacles.

A single mechanical stimulus was usually much more effective than electrical stimulation in inducing intense luminescence (*cf.* "flare," below) and quick effector facilitation.

The spatial uniformity of facilitation in the rachis was investigated in winter pansies, using two electrode pairs 22 mm apart. With repeated near-threshold stimuli delivered alternately at two second intervals, starting with electrode pair A, the first response most frequently occurred at the second shock—*i.e.*, at electrode B. Thereafter wave intensity built up stepwise for the first 6 to 8 responses just as if the successive stimuli were all being applied at one site. It was also possible to facilitate the rachis by repeated stimulation through one electrode and then, maintaining the same cadence, to obtain a response to the first shock delivered through the other electrode pair, 22 mm distant, rather than on the 2d to 4th, as is usual with non-facilitated rachides. Many such tests on a number of colonies are summarized in the first data line of Table I. The evidence that the rachis has been facilitated uniformly (92 responses on the first trial after switching, out of 107) is very strong, particularly in view of the possibility that some of the failures to respond (last column) were due to inadequate contact of the second electrode pair with the rachidial surface. However, in 5 of the 92 instances in which response to electrode B was immediate, luminescence only continued for a few additional cycles, as if net-wide sensory adaptation had already been nearly reached during stimulation via electrode A.

In 44 series stimulation through one electrode was continued until the rachis had ceased to respond (second data line of Table I), then the current was switched to the other electrode pair. In 8 of the 28 experiments of this group in which there was a response it occurred after the first shock. This result suggests that sensory adaptation in such instances was local. The average interval between the last response at electrode A and the time of switching electrodes was 21 seconds, the longest being 36 seconds.

In several hundred records of serial stimulation of summer pansies the number of the shock inducing the first visible response varied between 2 and 24. The number was seemingly less related to frequency or strength of stimulation than to "physiological state" of the colony. Similarly the number of stimuli required to reach fully facilitated luminescence varied over a wide range, some colonies reaching the plateau level in as few as 3 to 8 cycles whereas others might still be increasing in brightness after 20 successive stimuli (Fig. 7a) and still others might show hardly any increase in brilliance during long stimulation series.

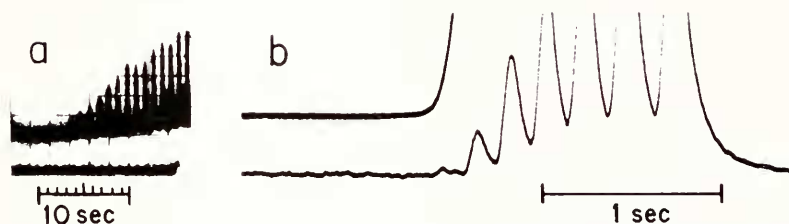


FIGURE 7. Oscillographic records of responses of *Renilla* to electrical stimulation. (a) Eighteen (?) successive responses to serial stimuli delivered at 1/sec; light trace above (left to right), stimuli below; sweep 2 secs/cm division; first response apparent after third shock; (b.) Response to stimuli delivered at 5/sec; upper trace, light detected from large area of rachis; lower trace, light detected from small area through $1\frac{1}{2}$ mm light guide.

The duration of the facilitated state was variable also. The most unequivocal measure would appear to be the length of time during which a particular mode of repetitive stimulation can be suspended before the rachis fails to respond at once when stimulation is resumed. Nearly a hundred such tests were made on fully facilitated winter pansies, with the result that 3 shocks at a frequency of one every two seconds, and 6 at a frequency of one per second, were the largest numbers that could be skipped with confidence that the colony would respond to the first subsequent shock. The time seemed little influenced by either the intensity of the shock or by the brightness of the luminescence prior to the skipping. Facilitation induced by moderate stimulation thus appeared to persist for approximately 8 seconds, or possibly as long as 10 if we accept the infrequent responses after 4 and 7 skips, respectively, at the two frequencies. This estimate is thus only a half to a third that obtained in the eight local adaptation experiments (Table I).

The effects of repetitive stimulation in relation to facilitation and adaptation will be considered below in further detail, but it is appropriate here to mention response limits. The highest frequency at which a rachis could usually be driven for more than a few cycles was about 3/second. Beyond this level the colony might become autoexcitable (q.v.), or refractory or appear to give light con-

tinuously for a short time ("flare"). In this last connection, photometric recordings through small holes showed, as expected, that the luminescence cycles of limited regions of the rachis were shorter than the integrated record of the whole rachis wave. This point is also well illustrated in a two channel recording in which the light emitted by a small area of a rachis being stimulated 5 times per second was detected through a light guide (Fig. 7b). This recording shows that although the rachis appeared to the eye, and in the integrative recording, to be tetanized, some of the effector tissue, at least, was responding 1:1 and showing facilitation in addition to some apparent summation.

Colliding waves. If the rachis was stimulated at the same time at opposite points on the margin, two simultaneous waves were induced which swept across the rachis and canceled each other upon meeting. This resulted in a curious "quadrille" effect in which two normal convex-forward waves advancing centripetally along one axis (Fig. 6e) transformed into two concave-forward waves traveling centrifugally at right angles to the original direction (Figs. 6f, 6g). Corresponding cancellations were observed when three or more wave-generating centers were active (v.i.).

TABLE 1

Experiments on spatial extent of facilitation. Colony first stimulated to plateau brilliance through electrode pair on one side of the rachis (usually at 30 shocks per minute), then by a pair on the opposite side, maintaining the cadence of stimulation. (Summary of tests on many colonies)

	Response at B to first shock after switch from A	Response to 2d or subsequent shock after switch	No response after switch
Responding 1:1 before switch (107)	92	6	9
Refractory before switch (44)	8	20	16

Extra and skipped waves. Vigorous pansies frequently showed another type of response, particularly during long-continued rhythmic stimulation at somewhat above threshold strength. In this response a wave originated at the electrode at the usual interval after stimulus, but was followed, before the next stimulus, by a second wave, also originating at the electrode, but much brighter. When the next stimulus occurred it might also elicit a brighter-than-normal response wave, or none at all. One may have, then, in the midst of a regular response series, either a "repeat" wave involving two waves abnormally bright and abnormally close together in time and space, the second occurring at normal response time and originating at the same site, or a single, brighter "extra" wave not at the normal response time, with the next normal response being skipped. In pansies that had been responding serially for a long time, waves were also skipped without warning, the wave immediately preceding such a skipped response seeming to be normally bright. Sometimes, however, such a skip was preceded by one or more waves of less than average brightness.

Reverse wave. In vigorous, low-threshold pansies stimulated on one side of the rachis every two seconds at somewhat above threshold intensity the wave of luminescence sometimes spread across the rachis and disappeared as usual,

(Fig. 6c) but was then followed, after a very brief delay, and before the next stimulus, by a brighter wave traveling in the opposite direction (Fig. 6d). This "reverse" wave originated at or near the point where the luminescence of the normal wave had died out, *i.e.*, usually at the point most distant from the original point of stimulation, and had the appearance of having been reflected back. Like the extra waves discussed above, a reverse wave might be followed, at the next stimulus, by either a brighter-than-normal wave from the electrode site or by a skipped wave.

Since excitation is known to traverse nonluminous parts of the colony such as the peduncle it seemed possible that the reverse wave represents a response to excitation that has spread from the electrode in the opposite direction from that in which the wave moves, passing around via the under surface of the rachis to the opposite edge, where the re-excitation of siphonozooids occurs. This possibility

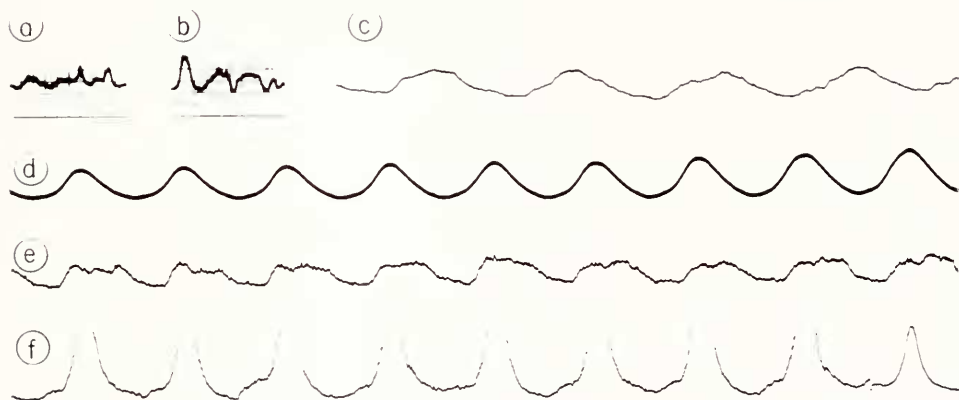


FIGURE 8. Oscillographs of *Renilla* luminosity during frenzy; a, b, slow sweep integrative records from large rachidial area 500 milliseconds between vertical grid lines; c, d, e, f, waveforms of colony luminescence in different stages of frenzy; wave frequency $\frac{1}{4}$ seconds in c; 1/second in d, e, f. Gain reduced for last wave of Figure 8f. See text.

was investigated by cutting a 3 mm strip off the margin of the rachis for half its circumference, presumably thus interfering with the continuity between possible subepidermal nerve nets on top and bottom surfaces of the rachis. However, when such rachides were stimulated at points opposite to the cut margin, typical reverse waves still occurred.

Volley or familial waves. With a single mechanical stimulation such as the initial contact with the electrode, a volley or family of waves was often induced rather than a single wave. Such an episode began with very brilliant waves in such quick succession that several were on the rachis simultaneously (Fig. 6h) but "ran down" or defacilitated in the course of a few seconds, the individual waves decreasing rapidly in brilliance and the inter-wave intervals increasing progressively. The contrast between the brilliant response to mechanical stimulation and that elicited by moderate electrical stimulation was especially striking in colonies that were fatigued or had acquired the high threshold and characteristically dim luminescence induced by long sojourn in stale water. Occasionally such

familial volleys were followed, at intervals of 10 seconds or more, by one or more additional waves, bright, and sometimes in the reverse direction. A similar phenomenon was often seen at the end of a long series of strong shocks at frequencies above the limit of 1:1 response. Here, after stimulation ceased, there originated from the electrode contact point a half dozen or more flashes of progressively decreasing frequency and brilliance. Rarely, a wave even occurred a minute or so after stimulation and luminescence had ceased, raising the question of whether it represented a long-delayed response to the original excitation or was truly spontaneous.

Frenzy. The most extraordinary type of light emission in *Renilla* is one which I shall call "frenzy" and whose appearance is suggested by terms in my tape-recorded descriptions: "sputtering," "rippling," "circling," "oscillating," "boiling," "pinwheel," "propeller." The common features of these bewildering manifestations were long-continued autoexcitation and a constantly shifting point or points of wave origin. Not only was the activity spontaneous but, once well started, it seemed relatively independent of environmental influences. If the electrode was left in position (with the stimulator turned off) the contact point sometimes appeared to be a point of origin of some of the spontaneous waves, but excitation continued equally well with nothing touching the rachis.

A simple type of frenzy, with two alternating excitation centers was that diagrammed in Figure 6m, where the wave rocked back and forth across the rachis, the excitation centers progressing around the margin. In "boiling," waves spread out radially from two or more excitation centers active either synchronously or asynchronously, canceling on impingement (Figs. 6i-6l). Occasionally the luminous waves became phased for a short time so as to give a continuously rotating effect like a two or three bladed propeller (Fig. 6n). Figures 6o-6q, illustrate how multiple centers and wave "reflection" combined to create concave-forward waves and a whiplashing impression. In all these behaviors there was an accompanying lasting glow of autozoid calices which tended to fill in the intensity troughs between the waves and make photometry difficult, though the eye was able to sort out the siphonozoid activity.

With the rachis constantly alight at one point or another it was difficult to make accurate comparisons with normal waves, but the propagation rate of the fragmented waves during frenzy appeared normal and did not change with time. By concentrating attention upon a single small area of the rachis it was seen that although the region was occasionally dark for several cycles, a fairly regular rhythmicity underlay the apparently highly irregular activity. The frequency of this spontaneous auto-excitation was consistently about 37/min at 21°. Figures 8a and 8b illustrate the irregular fluctuations in rate of total light output from the rachis that often prevailed, whereas Figures 8c-8f show the rather regular rhythmicity that obtained at other times.

Frenzy might continue for only a few minutes or persist for more than an hour. It ordinarily involved considerably brighter waves than are usual, for example, with one electrical stimulus every two seconds. However, particularly in winter pansies that had (it seems) exhausted themselves in an initial burst of activity due to mechanical contact of the electrode, the luminescence might be very dim. In general the average luminous intensity decreased slowly with time, but in several instances in which cessation of frenzy was actually observed the luminescence was still moderately intense at that time. In numerous pauses in

which a bout of frenzy lasted only a few minutes it was readily re-excited and proceeded again at full intensity. On the other hand, a pansy that had gone through a long frenzy was often not re-excitabile until it had had a rest period.

The conditions which induce frenzy are discussed in more detail below, but for present purposes it can be said that the phenomenon was more characteristic of vigorous colonies than of those with high thresholds or dim luminescence and that it could usually be induced by strong, long-continued repetitive electrical stimulation at frequencies of 30–45 per minute or higher.

Flare. A phenomenon not infrequently seen with initial mechanical or strong electrical stimulation of summer pansies, and commonly seen in winter pansies, was a brilliant even glow which lasted for a brief period at apparently constant intensity and then died away over as long as 30 seconds. Though such flares usually showed no trace of waves the light was often not absolutely uniform but gave the impression that the individual luminous points over the rachidial surface (siphonophore clusters) were pulsing or scintillating at high frequency. In a few instances a flare resolved into familial waves.

An interesting feature of glows and flares was that by repetition of the precipitating stimulus the light could be "pumped up" repeatedly to peak brilliance.

Autozoid glow. The long-lasting autozoid glow which often developed during serial stimulation could become quite bright but was always dimmer than the siphonozoid waves that occurred at each stimulus and was coarser-grained. The relation between persistent interwave glow and the flash is not simple. Glow was observed most often in bright, vigorous pansies but occurred also in stale colonies. Similarly, both low-threshold, dark-adapted pansies and light-adapted pansies sometimes glowed, and glows were seen together with either narrow waves or broad. The speed of development of glow also varied, some pansies still showing complete extinction of luminescence between waves after 50 or even 100 successive responses whereas in others a glow might develop with fewer than 10 successive stimuli. Glows were most frequent with stimulation well above threshold strength and with frequencies higher than the standard 30 min, but some pansies developed autozoid glow at threshold strength and low frequency.

Glows occurred after familial waves and during frenzy, indicating that spontaneous excitation as well as applied stimuli was effective. One consistent generalization is that the threshold for glowing was at least as high as for flashing; Glows never developed at stimulus strengths below that required to induce waves and as soon as stimulus strength was reduced below the threshold for waves any glow that might have developed began to fade.

The glow was generally uniform over the rachis. Occasionally it seemed more pronounced at the margins, an effect that could be due either to the greater concentration of zooids peripherally or to the more tangential view along the down-curving surface of the margin. Persistence of glow was quite variable, being usually of the order of 20 seconds after cessation of stimulation but not uncommonly differing from this by a factor of 2 in either direction.

Interrelations of behavior types

Though some of the different types of light emission in *Renilla* seemed to depend on particular modes of stimulation, their interrelations were not immediately

obvious. In order to clarify this matter, stimulus intensity, frequency and number were explored systematically. Ability to follow 1:1 through a series of 10-15 consecutive stimuli was tested at each of several intensity-frequency combinations and if possible each series was repeated several times with each pansy. Also whenever possible the effects of stimulus strength and frequency were tested separately by facilitating the colony at one set of stimulus parameters then changing either the intensity or frequency. Long term behavior was also explored. Development of frenzy was an obstacle to repetition in many tests, particularly with high stimulus frequencies and strengths.

TABLE II

Relation between strength of stimulus and ability to give 1:1 response to a given frequency of stimulation. The categories of response ("grouped," "failing," etc.) are defined in the text. The number following the category name is the number of series of at least 10 successive stimuli in which the indicated type of response was observed

Nominal strength (V)	30 per minute	60 per minute	120 per minute	180 per minute
4	response	response 6 failing 3 grouped 6 alternating 1	grouped 4 alternating 1	single 4 failing 1
16	response	response 16 failing 8 grouped 1	response 18 failing 20 alternating 4 frenzy 3	failing 14 grouped 3 alternating 1 frenzy 2
72	response	response 7	response 16 failing 11 frenzy 2	response 10 failing 14 alternating 2

Table II summarizes several hundred tests with 12 combinations of stimulus strengths and frequencies. All pansies that responded to stimulation at 30 per minute were able to maintain response for 10-15 cycles at all voltages tested. At higher frequencies there were also successful series, particularly with the higher voltages, but there was an increasing percentage of breakdowns of several kinds. In "grouped" responses a few consecutive skips alternated with a few consecutive responses. In "alternating" behavior the colony skipped every other response, or sometimes two out of three potential responses. In the "failing" category the pansy started out apparently responding in perfect correspondence with the stimulation frequency but gradually fell behind and by the 10th consecutive wave or so was out of phase by a third or half cycle. If stimulation was then continued the responses became "grouped" and then more and more irregular until they ceased altogether.

There was, of course, considerable difference between individuals, but a number of definite trends were observed. First, ability to follow a given frequency was definitely greater the greater the stimulation intensity. This is well shown by the series made with 3 shocks per second, in which at threshold strength the almost invariable

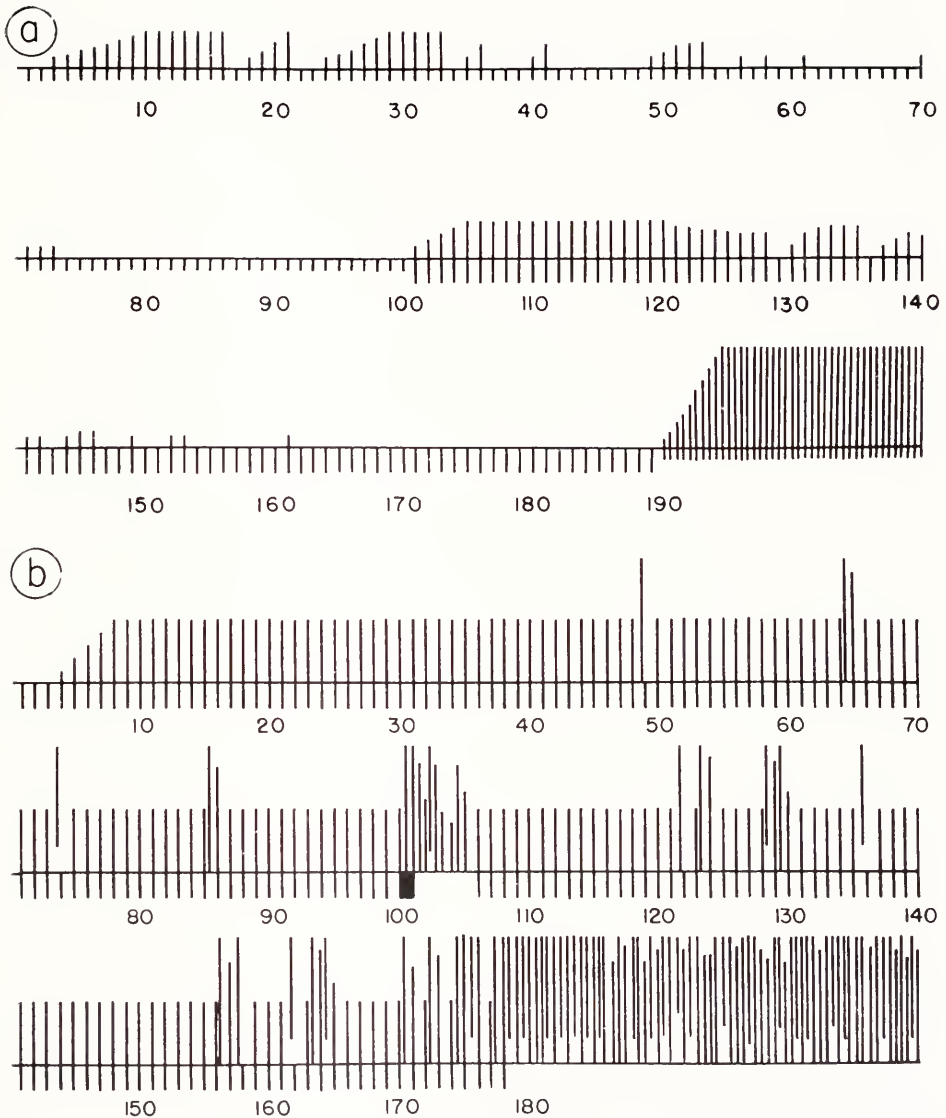


FIGURE 9. Diagrams of long records of response of *Renilla* colonies to serial electrical stimulation. Wave intensities indicated by lengths of vertical lines above horizontal axis. Relative intensities and frequencies of stimulation indicated by lines below horizontal axis. No attempt is made to show latencies. "Reverse" waves suggested by vertical lines not reaching horizontal axis. (a.) One hundred successive stimulations at threshold strength (4 V) and frequency of 30/min are followed by 89 stimuli at increased voltage but same frequency, and then by 41 stimuli at 4 V and 1/sec. Facilitation indicated at episodes 3-10, 24-33, etc.; skips at 17, 21, 22, etc. (b.) One hundred successive stimulations at 16 V and frequency of 30/min are followed by mechanical stimulation (eliciting "extra" and "reverse" waves), then resumption of original stimulations; leading to autoexcitation (frenzy) by about episode 178. Brighter extra waves indicated just after responses 64, 85, etc., followed by facilitated waves; brighter waves just after responses 48, 121, etc., causing skipping; reverse waves at episodes 73, 136, etc.

response was a single flash, whereas at a strength of 72 V. or nearly twenty times the threshold stimulus strength, 1:1 responses were obtained in 10 of 26 trials. Secondly, performance tended to deteriorate with repetition. For example, a pansy might follow perfectly for 10 shocks on the first test at 60 per minute and threshold strength, but fall behind in successive trials in spite of intervening periods at 30 min (which was always followed without difficulty). Thirdly, the "grouped" type of response seemed characteristic of the lower frequencies and strengths of stimulation, whereas failure to maintain response at higher frequency-strength

TABLE III

Five representative stimulation series at 30 stimuli per minute which terminated in frenzy. Numbers in all but first three columns are stimulus sequence numbers at which indicated event occurred

Exp. No.	Stim. Str. (V)	First Response Start Shock	Extra, with brighter normal	Extra, with skip	Reverse with brighter normal	Reverse with skip	Frenzy	Remarks
1	16	3			51*, 143*, 182, 200, 229, 235, 238	117	258	225, dimmer; 258, two centers active
2	40	3	8, 57**	37			165	100, dimmer
3	16	2	70*	277†		175†, 256†	280	83, two simultaneous waves from different points
4	16	4	78, 95**, 240*		154, 164, 323, 345	288	377	100-150, dimmer
5	16	2		300		102, 261, 271, 279	375	332, skipped; 100-180, brightest; 338-350, dim

* Not much facilitation of next normal wave.

** Extra wave of normal brightness.

† At proper time for normal wave.

‡ From second center.

combinations more usually took the form either of complete refractoriness or alternation, and it was of course also in this region that the response was most likely to pass over into frenzy.

Within limits, intensity of luminescence increased with increasing frequency of stimulation. Stepwise increase in brilliance as the pansy responded to the new frequency indicated that facilitation was involved, and a reverse or "defacilitatory" process was seen when stimulation frequency was suddenly reduced. If stimulus strength was suddenly increased, even as much as 10-fold, during the plateau phase of a response series, no increase in luminescence was observed. The effect of stimulus strength in maintaining response in long serial stimulation (overcoming adaptation) has already been mentioned.

Long-continued stimulation usually induced progressive changes in luminous behavior leading either to failure of response or to autoexcitation. Failing response, which was common with mild stimulation, is considered due to "adaptation" rather than to junctional or effector fatigue both because, in some series with higher voltages, many hundred successive responses could be induced and because very prolonged periods of spontaneous flashing occurred during frenzy. Adapting series might terminate without warning but ordinarily involved a few sporadic and short-lived resumptions of response after the initial failure. Frenzy, on the other hand, developed via a variety of the spontaneous behaviors already discussed. Table III summarizes five actual series, with some indication of intensity fluctuations as well as my interpretation of successive episodes. Composite diagrams to illustrate typical development of adaptation and of frenzy, as recorded from visual observation, are given in Figures 9a and 9b.

Siphonozooid activity during rachidial responses

Though records of luminescent waves delimit roughly the activity cycle of the siphonozooid clusters they give only a statistical view of the frequency of participation of the individual clusters and the temporal coordination of their activities. Visual observations of such phenomena must always be somewhat questionable because of the difficulty of maintaining fixation between flashes, but from microscopic viewing of many hundred waves during both electrical stimulation and frenzy, the following tentative conclusions were drawn: (a) a given cluster participated in each successive wave, though not always at the same brightness, (b) different clusters could be of quite different peak brilliance, (c) the increase in intensity of light emitted during facilitation was due to increase in the activity of the individual clusters, not to activation of additional clusters interspersed among those originally responding, (d) not every individual siphonozooid in a cluster necessarily produced light at every stimulus, (e) not all the individual siphonozooids of a cluster were of equal brightness, (f) the brilliance of a given individual siphonozooid could vary from wave to wave. From the fact that each wave was brightest at its leading edge it was deduced (g) that the ultimate unit response is asymmetrical—i.e., has a relatively more rapid rise phase than decay phase.

Though *Renilla* colonies characteristically responded as a whole, and excitation is thought to affect all parts of the rachis equally, sometimes a few points of light were seen in response to the first one or two shocks of a strength which proved to be below threshold for the rachis as a whole, thus suggesting that the thresholds of the individual zooids or siphonozooid clusters differed somewhat. Similarly, when a rachis had become adapted and no longer responded as a whole, or had become fatigued by frenzy, a few zooids sometimes continued to light up in apparent response to the passage of a wave of excitation to which the rest of the zooids were refractory. Also, individual clusters occasionally flashed spontaneously in pansies that had not been stimulated for several minutes, perhaps indicating the attainment of local autoexcitatory states.

Mechanical injury sometimes elicited local luminescence, such as shown at the electrode site in Figure 5, which persisted steadily for many minutes. An interesting and unexplained phenomenon is that a single siphonozooid of a cluster, or a single lobe of an autozooid calyx, occasionally remained lit up steadily through

several to many seconds, apparently independent of injury, general rachidial waves or stimulation, and with a brilliance at least equal to that of any point on the rachis during passage of a normal luminous wave.

One further type of luminescence, sometimes seen briefly after mechanical stimulation, gives the impression of scintillation—*i.e.*, as if many of the siphonozoid clusters were sparkling rapidly and out of phase with each other and independently of the usual wave luminescence or indeed of any rachis-wide coordinated excitation.

DISCUSSION

Assuming that the spread of luminescence over the *Renilla* colony mirrors the paths of excitation in a nerve net, the types of behavior described above might permit an analysis of net behavior hardly attainable from any other material. Some conclusions, particularly in relation to autoexcitability, are already apparent and are discussed below. Other very suggestive indications would be strengthened by a more critical discrimination between conductional, junctional and effector roles, and by better knowledge of the excitation cycles of the individual polyps and their parts, such as could be obtained by local stimulation. Preliminary results from such experiments (Buck and Hanson, 1967) were so promising—for example, in disclosing the existence of non-propagated responses and differing latency classes—that most of the discussion of nerve net physiology and theory, and the relation of the work on *Renilla* to the extensive literature derived from work on other coelenterates, have been deferred to a subsequent paper (Hanson and Buck, in preparation).

The data for polyp population (Fig. 4) and gross conduction velocity permit estimation of the minimal extent of the putative nerve net. The facts that the waves spread evenly, with the leading edge a smooth arc and with no sign of preferred direction relative to rachidial geometry, require siphonozoid interconnections in at least six directions. Assuming isolateral triangular connectivity and equal distance between clusters, and considering the rachis a hexagon of equivalent area, the number of siphonozoid clusters and the lengths of their interconnections are related by the formula $N = 3d(d + 1) + 1$, where N is the total population of siphonozoid clusters and d is the number of unit distances (cluster-to-cluster) along one edge of the rachidial hexagon. Substituting 1500 for N and solving for d , the corresponding hexagonal rachis works out to be about 22 connectivity units on an edge, or 44 units for a diameter (vertex to para position vertex). Using 6.6 cm as a reasonable actual diameter, the unit cluster-to-cluster distance is therefore about 1.5 mm. Assuming that each siphonozoid cluster transmits excitation to its three nearest centrifugal neighbors, and neglecting all autozoid connections, the total length of net involved during passage of a wave over a 6.6 cm colony would be not less than $1500 \times 3 \times 0.0015$, or about 6.7 m.

The differences between "summer" and "winter" colonies, whatever their cause and generality, are interesting and valuable from several aspects. If summer pansies had been used exclusively I would certainly have concluded that rapid autozoid withdrawal and rachidial wave luminescence are coupled responses. I was not able to tell whether the startle reaction can occur without luminescence but the fact that autozooids in winter pansies sometimes did not withdraw after excita-

tion strong enough to elicit luminescence from the whole rachis (including the autozoid tentacles) shows that muscular and bioluminescent responses are not in obligatory linkage. This does not mean that the two systems necessarily require separate conduction pathways, since differing requirements for facilitation could divorce the responses. Neither do my observations prove that rapid withdrawal, when it does occur, is triggered by the same excitation that induces flashing, though the two events often occur simultaneously. Parker (1919, 1920a, 1920b) considered the rapid withdrawal to be mediated by a different system from the slow rachidial peristalsis.

A second deduction from winter pansies is that at least the tentacles of the autozooids are connected to the general rachidial net that transmits excitation to the siphonozoid cluster. My inclination to believe that the trunk or column of the autozoid is non-luminous, or at least far dimmer than the tentacles, accords with Parker's conclusion from crushed autozooids, but since his treatment did not elicit light from the tentacles either, the matter must be left in abeyance. The variability observed in the laboratory reinforces the need for behavioral observations in the native habitat, particularly in connection with the questions of whether the colonies luminesce spontaneously, whether the autozoid tentacles participate regularly in rachidial luminescence along with the siphonozooids, whether there is autozoid withdrawal whenever a wave is generated, and whether autoexcitation occurs in nature.

By poking the white "granules" around the autozoid (the calyx lobes) Parker (1920b) discovered that the calyx is luminous but did not realize that the tissue also luminesces in response to distant stimulation of the rachis. Actually, the behavior of the calyx stands in curious contrast to that of both the autozoid tentacles and of the siphonozooids. The fact that no autozoid luminescence is usually seen in summer pansies during rachidial waves evoked by low frequency electrical stimulation that is near the threshold for siphonozoid responses could be attributed to the calices having a systematically higher threshold than the siphonozooids, or lower brilliance, or both, but the much greater sluggishness of the luminescence, when it does occur, suggests the possibility of a different effector tissue type or differing mode of excitation. On the other hand, the facts that calyx luminescence does augment in parallel with siphonozoid luminescence during vigorous repetitive stimulation, and that it does appear during frenzy, argue for excitation *via* the primary net.

The indication that luminescence in *Renilla* is intracellular is somewhat surprising, for although this is usual in coelenterates, secretion of a luminous slime was seen in the closely related sea pen *Cavernularia* (Harvey, 1917).

Cancellation of light emission when waves collide (Fig. 6e-6g) was observed by Panceri (1872) and Moore (1926) in *Pennatula*. The phenomenon was ascribed by Moore to a refractory state in the nerve net behind the advancing wave of luminescence that persists long enough to block conduction in the opposite direction. This seems a reasonable explanation and seems also compatible with the phenomenon of skipped waves during serial stimulation and with the origin of the "repeat" category of spontaneous waves from the site of electrode contact, the region of the rachis that has had the longest time to recover. However, Moore's

hypothesis is difficult to reconcile with "reverse" waves (Fig. 6c, 6d), which appear to originate from the region of the rachis most recently excited.

The virtual absence of decrement in the spread of luminescence in *Renilla* argues against the presence of facilitation-requiring synapses in the hypothetical nerve net itself, as does the fact that luminescence spreads evenly and apparently with equal facility in any direction. The presence of well-marked stepwise augmentation of luminescence, however, would argue strongly for the presence of neuroeffector junctions if it could be proven that this phenomenon is not due primarily to recruitment of additional luminous units. Insofar as I can judge visually, recruitment is not a major factor—hence my use of "facilitation"—but the necessity for periodic interruptions of view make it impossible to be sure. Ability to produce stepwise augmentation of luminescence by stimulating at two sites alternately, and to obtain a response to the first shock applied at one point after repetitive stimulation at another, provide additional evidence for net-wide facilitation, as does the persistence of a ready state for up to perhaps 30 seconds.

There is no reason to suppose that frenzy, a consequence of violent or repetitive stimulation, is a normal response for *Renilla* in its native habitat. Nonetheless, analysis in terms of combinations of autoexcitatory (spontaneous) behaviors such as repeat and reverse waves, with collision cancellation and facilitation, brings some order to an otherwise bewildering phenomenon. The existence of multiple and shifting autoexcitatory centers is a secondary complication to the primary problem, which is the persistence of spontaneity long past the time when any hyperfacilitation or after-discharge would be expected to have died out. The possibility of continuing excitation by a trapped circuit wave of the sort seen in the scyphozoan sub-umbrellar nerve (Bullock, 1943) is attractive, particularly during frenzies in which the luminescence actually does circle the perimeter of the rachis. Conceivably the constantly shifting pattern of spread and the appearance of luminescence in the most recently active regions (reverse waves) will turn out to reflect excitation that is circling the rachis vertically, so to speak, rather than horizontally, via the non-luminous under surface or via some internal pathway. The rather constant natural frequency of about one/sec at 25° for frenzied flashing is reasonably close to the conduction delay expected for circuits of that length, although my experiments involving trimming the rachidial margin did not support the idea of net connections via non-luminous parts.

My visual observations and Nicol's (1955a, 1955b) recordings confirm and complement each other in most of the aspects of *Renilla* luminescence that we both studied. In certain matters where there appears to be some disagreement—for example, in our respective estimates of the persistence of facilitation (Nicol, 10 minutes; Buck, 36 seconds), in the extent of sensory adaptation over the rachis (Nicol, uniform; Buck, sometimes local), in the involvement of recruitment in the augmentation of luminescence (Nicol, postulated; Buck, not visible)—it is possible that differences in techniques may be involved. Ordinary photometric recordings from any considerable portion of the rachis (*e.g.*, Figs. 8a–8f) can be unreliable indicators of siphonozooid kinetics during the passage of a wave, even in regard to overall waveform (Buck, 1955), and even when intensity changes with time are not further obscured by sustained glowing of autozoid calices. Recordings made through a light guide (Fig. 7b) indeed show that small areas may

have a much shorter cycle than the mass wave though they do not identify the sources anatomically. What is needed here, as in other connections discussed earlier, are the data derived from individual stimulation of single zooids (Hanson and Buck, in preparation).

Most of this work was done at the Kerekhoff Marine Laboratory of the California Institute of Technology during tenure of a Visiting Professorship and in the laboratory of Professor T. H. Bullock at the University of California, Los Angeles. Special thanks are also due George and Nettie MacGinitie, George Beadle, T. D. Coyle and J. F. Case for valuable assistance. Numerous colleagues have ministered to the manuscript during its long gestation and Dr. Nicol is particularly thanked for his generous acknowledgment of its content. The photographs for Figure 1 were taken by Professor MacGinitie. The oscilloscope records used for Figures 7 and 8 were made at the National Institutes of Health in collaboration with Dr. Frank Hanson, and the image intensifier photographs of Figure 5 were made at Princeton University by Dr. Hanson in collaboration with Professor George Reynolds. Figure 4 was prepared by Edith Bidwell.

SUMMARY

1. Some details are given of the external morphology of the autozooids and siphonozooids and of their distribution in the colony. It is estimated that a minimum of over 6 meters of nerve net would be required to conduct excitation across an average-size colony during the passage of a wave of luminescence.

2. An account is given of the localization of luminescence in the two types of polyp and of their apparently differing behaviors in colonies collected in summer and winter.

3. In summer colonies the sharp luminous waves induced by electrical stimulation are entirely due to siphonozooids. Under strong stimulation the autozooid calices produce a long lasting glow.

4. Neuroeffector facilitation takes place uniformly throughout the colonial conduction system. Decay of facilitation requires 10–36 seconds, by different tests. There are indications that sensory adaptation in the (hypothetical) net can be local.

5. Local recording shows that the response cycle in small areas of the colony is much shorter, and its frequency response much higher, than indicated by integrative recordings of the wave response as a whole.

6. Individual siphonozooid clusters can flash repetitively in successive waves, fail to participate in every wave and vary in intensity from wave to wave. The increase in light intensity during successive facilitating waves seems due to increase in the activity of individual clusters, not to recruitment of additional clusters. There were indications of individual differences in threshold, adaptation and auto-excitation between clusters.

7. During strong repetitive electrical stimulation there may arise extra siphonozooid waves of augmented brightness, running in the same direction as the "normal" waves (*i.e.*, centrifugally from the electrode) or in the reverse direction.

colony may then enter an autoexcitatory state ("frenzy"), independent of external stimulation and often involving development of several excitation centers, in which waves of irregular and constantly changing form course over the rachidial surface for up to an hour.

LITERATURE CITED

- AGASSIZ, L., 1850. On the structure of the Halcyonoid polypi. *Proc. Amer. Ass. Advan. Sci.*, **3**: 207-213.
- BUCK, JOHN, 1953. Bioluminescence in the study of invertebrate nervous systems. *Anat. Rec.*, **117**: 594.
- BUCK, JOHN, 1955. Some reflections on the control of bioluminescence. Pages 323-333 in F. H. Johnson, Ed., *The Luminescence of Biological Systems*. American Association for the Advancement of Science, Washington, D. C.
- BUCK, JOHN, AND FRANK E. HANSON, JR., 1967. Zooid response in *Renilla*. *Biol. Bull.*, **133**: 459.
- BULLOCK, THEODORE H., 1943. Neuromuscular facilitation in scyphomedusae. *J. Cell. Comp. Physiol.*, **22**: 251-272.
- HARVEY, E. NEWTON, 1917. Studies on bioluminescence. VI. Light production in a Japanese pennatulid, *Cavernularia haberi*. *Amer. J. Physiol.*, **42**: 349-358.
- HYMAN, LIBBIE HENRIETTA, 1940. *The Invertebrates. I. Protozoa through Coelenterata*. McGraw-Hill, New York, 726 pp.
- KREISS, PAUL, AND MILTON J. CORMIER, 1967. Inhibition of *Renilla reniformis* bioluminescence by light. Effects on luciferase and its substrate. *Biochim. Biophys. Acta*, **141**: 181-183.
- MACGINITIE, G. E., AND NETTIE MACGINITIE, 1968. *Natural History of Marine Animals*. [2d Ed.] McGraw-Hill, New York, 523 pp.
- MOORE, A. R., 1926. On the nature of inhibition in *Pennatula*. *Amer. J. Physiol.*, **76**: 112-115.
- NICOL, J. A. C., 1955a. Observations on luminescence in *Renilla* (Pennatulacea). *J. Exp. Biol.*, **32**: 299-320.
- NICOL, J. A. C., 1955b. Nervous regulation of luminescence in the sea pansy *Renilla köllikeri*. *J. Exp. Biol.*, **32**: 619-635.
- PANCERI, PAUL, 1872. The luminous organs and light of the Pennatulæ. (Transl.) *Quart. J. Microscop. Sci.*, **12**: 248-254.
- PARKER, G. H., 1919. The organization of *Renilla*. *J. Exp. Zool.*, **27**: 499-507.
- PARKER, G. H., 1920a. Activities of colonial animals. I. Circulation of water in *Renilla*. *J. Exp. Zool.*, **31**: 343-367.
- PARKER, G. H., 1920b. Activities of colonial animals. II. Neuromuscular movements and phosphorescence of *Renilla*. *J. Exp. Zool.*, **31**: 475-515.
- WILSON, EDMUND B., 1884. The development of *Renilla*. *Phil. Trans. Roy. Soc. London*, **174**: 723-815.

Note added in proof: Professor Reynolds has just published several interesting image intensifier photographs of *Renilla* luminescence (Reynolds, George T., 1972. Image intensification applied to biological problems. *Quart. Rev. Biophysics*, **5**: 295-347.)